CYTOTAXONOMIC STUDIES IN THE GENUS *URGINEA* STEIN IN WEST AFRICA. IV. POPULATION DIFFERENTIATION AND KARYOTYPE VARIATION IN *URGINEA INDICA* (ROXB.) KUNTH[1]

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ABSTRACT

Qualitative and quantitative studies of karyotypes of over 250 individual plants of the variable species *Urginea indica* (Roxb.) Kunth were carried out. The plants were sampled from 23 collection sites representing seven distinguishable phenotypes. Root tips were used for mitotic preparations. Ten karyotypes were recognized, four of which represented the first phenotype while the remaining six represented each of the other phenotypes. The species is aggressive in its exploitation of various ecological niches. Small, homogeneous, genetically distinct populations have evolved a response to the demands of each ecological niche and a device to isolate the individual gene pools.

Morphological variability is an undisputed attribute of a species composed of sexually reproducing individuals in a large panmictic population. However, the assumption that continuous populations were spatially fluid, panmictic, and genetically homogeneous has been assailed by the results of extensive works on both plants and animals (Epling & Dobzhansky, 1942; Selander et al., 1969; Bradshaw, 1972; Jones, 1973; Schaal, 1975). This assumption has now been largely replaced by the theory that, particularly in plants, many extensive populations consist of numerous semi-isolated demes. This, according to Linhart et al. (1981), may be due to the effect of diversifying selection in heterogeneous environments and/or highly restricted gene flow as a result of spatial isolation. There is abundant evidence in support of each of these two phenomena in the process of speciation. Evidence of restricted gene flow has led to the assumption that, within continuous populations, there exist small clusters of genetically related individuals (Bradshaw, 1972; Levin & Kerster, 1974). In spite of this, the existence of distinct correlated discontinuities in the phenotypic characteristics of a continuous population, which thereby render the population morphologically heterogeneous, would subsume the existence of distinct segmentation in the genetic structure of the population (cf. Oyewole, 1971). Such segmentation can be maintained only by a number of factors, chief among which is an intrinsic isolation mechanism.

The present work analyzes the results of studies of the genetic structure of morphological variants of the variable species or species complex, *Urginea indica* (Roxb.) Kunth. The morphological differentiation among the large form is the subject of the third in a series of studies of the genus (Oyewole, 1986).

MATERIALS AND METHODS

Populations were sampled in the wild, and plants were cultivated in experimental sites (Oyewole, 1986). Collection sites are illustrated in Figure 1. The distribution of the species, between latitudes 7°N and 10°N, spans the deciduous woodland and savanna of the Southern Guinea Savanna vegetation zone. Over 250 plants were collected from 23 sites in 17 sampling areas. Plants from distinct populations were grown together under the same experimental conditions; seven distinct morphological groups were recognized. Four of the forms (A, B, C and D) represent the large form, while E, F and G belong to the dwarf form.

Root tips were harvested between 8 and 9 A.M., pretreated for one hour in sat. aq. 1,4-dichlorobenzene, fixed, and treated for mitotic squash preparations following the conventional methods (Darlington & LaCour, 1969). Chromosome counts were taken at random from every preparation. Chromosome measurements were taken from not less than 100 cells, at full mitotic meta-

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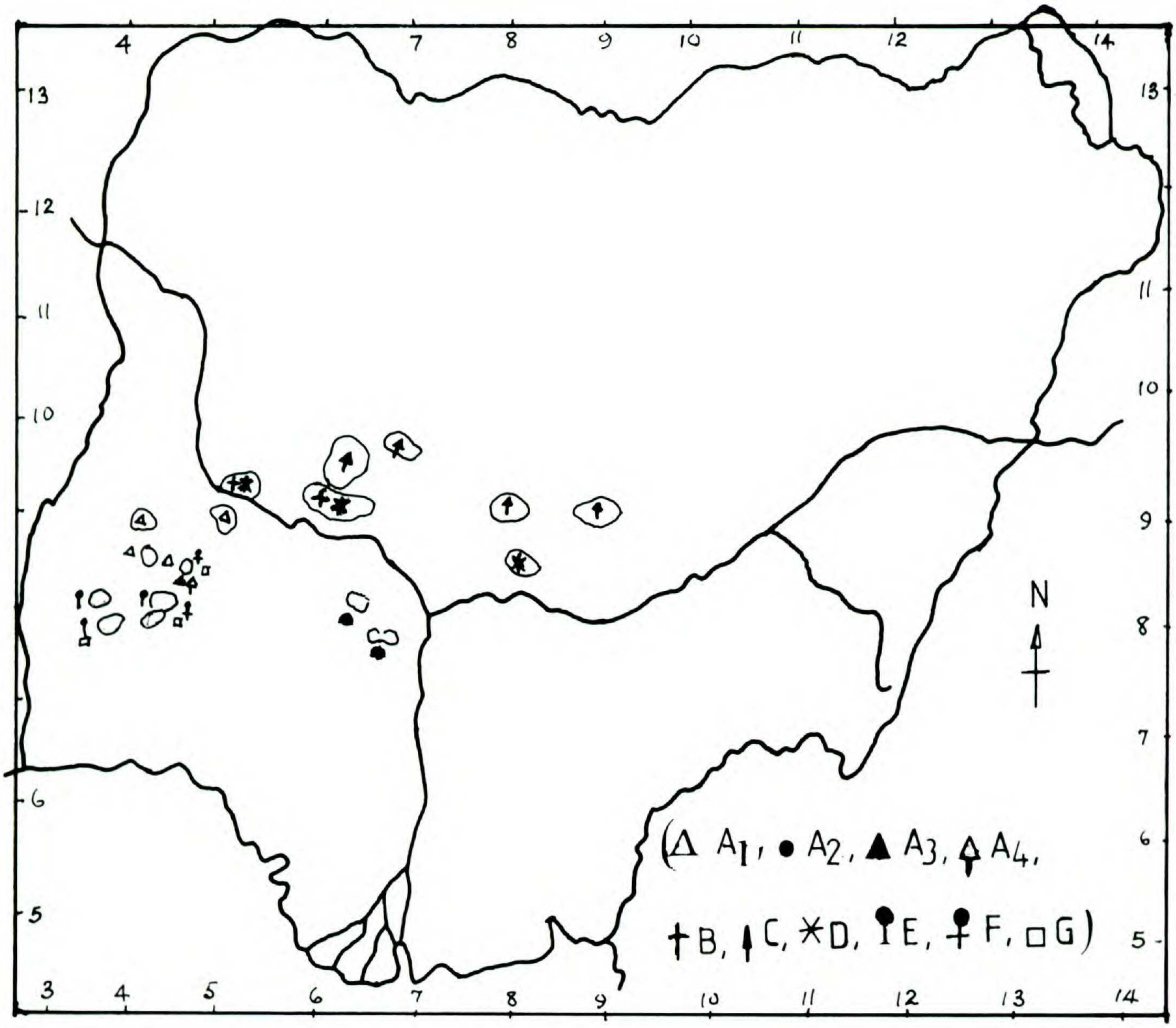

FIGURE 1. Map of Nigeria showing areas of major sampling sites.

phase, in each morphological group. Homologous chromosomes were easily identified in each set of measurements from comparative chromosome lengths and the relative lengths of chromosome arms. Measurements were recorded in order of magnitude for the haploid set. Data were pooled for each group, from which average chromosome length, the relative chromosome-arm length, and the position of the centromere were determined.

Flower buds of appropriate age were collected between 7 and 10 A.M., immediately incised and fixed, and the anthers squashed and stained. As many inflorescences as were available in each group were sampled, and meiotic stages from pachynema to telophase II were examined in not less than 100 pollen mother cells (PMCs) in those groups that flowered (not all the groups have flowered in cultivation).

RESULTS

Figure 2 contains somatic metaphase complements of the various groups. All the groups have $2n = 20$. Karyotype data is summarized in Table 1. Each morphological group is represented by a different karyotype. The total length of chromatin material, at metaphase, of each karyotype differs from the others. The karyomorphology is similar, although intrinsic differences abound (Fig. 3). Chromosome classification, using the chromosome index (ratio of long arm to short arm), is according to Levan et al. (1964). The chromosomes in each complement were classified as long (6.0 μm and above), medium (4.0–5.9 μm) and short (below 4.0 μm). Details of the meiotic study will be presented in a subsequent part of this series.

Group A. This group is represented by four

TABLE 1. Summary of karyotype data (*Urginea indica*).

Homologues		A_1	A_2	A_3	A_4	B	C	D	E	F	G
1	Chromatin length	11.0	10.56	7.5	7.5	9.5	13.1	9.82	13.5	12.05	12.33
	r value	10.0	9.06	6.5	6.5	8.5	11.25	9.67	11.5	11.05	8.25
	Centromere location	t	t	st	st	t	t	t	t	t	t
2	Chromatin length	10.0	9.88	7.25	6.88	8.5	11.75	8.5	11.5	9.35	11.33
	r value	9.0	8.88	8.67	17.3	10.3	10.75	7.5	10.5	8.8	12.6
	Centromere location	t	t	t	t	t	t	t	t	t	t
3	Chromatin length	8.0	7.5	4.88	5.0	6.13	9.56	7.16	9.25	8.63	8.81
	r value	9.67	7.0	8.75	9.0	7.2	8.56	7.6	11.33	7.85	10.9
	Centromere location	t	st	t	t	t	t	t	t	t	t
4	Chromatin length	5.5	5.5	4.75	4.0	5.75	7.13	5.0	7.0	7.8	6.82
	r value	6.0	4.5	5.33	5.6	6.67	7.14	9.0	6.0	7.67	7.74
	Centromere location	st	st	st	st	st	t	t	st	t	t
5	Chromatin length	5.0	4.75	3.5	4.0	4.75	6.0	4.5	5.63	5.65	5.69
	r value	9.0	6.6	6.0	7.0	8.5	5.86	5.0	6.5	6.5	5.4
	Centromere location	t	st	st	st	t	st	st	st	st	st
6	Chromatin length	4.5	4.63	3.5	3.5	4.5	5.92	4.17	5.25	5.43	5.4
	r value	8.0	8.25	6.0	6.0	5.0	7.88	8.07	6.0	6.0	5.59
	Centromere location	t	t	st	st	st	t	t	st	st	st
7	Chromatin length	4.0	4.31	3.0	3.0	3.88	5.5	3.92	5.25	5.28	4.96
	r value	7.0	3.31	3.6	1.0	4.2	5.29	4.88	6.0	6.3	5.36
	Centromere location	st	st	st	m	st	st	st	st	st	st
8	Chromatin length	4.0	4.06	3.0	3.0	3.67	5.25	3.92	5.13	5.15	4.71
	r value	5.0	4.41	3.2	5.0	7.74	5.0	4.88	7.2	5.0	5.63
	Centromere location	st	st	st	st	t	st	st	t	st	st
9	Chromatin length	4.0	3.68	2.75	3.0	3.5	5.13	3.75	4.5	4.55	4.45
	r value	7.0	6.36	2.5	5.0	5.14	7.2	4.0	5.0	6.6	5.29
	Centromere location	st	st	sm	st	st	t	st	st	st	st
10	Chromatin length	3.67	3.31	2.5	2.5	3.0	4.88	3.5	4.25	4.10	4.14
	r value	4.5	4.91	2.6	4.0	5.0	8.75	6.0	4.67	6.9	3.93
	Centromere location	st	st	sm	st	st	t	st	st	st	st
Total chromatin length/complement		119.34	116.36	85.26	84.76	106.36	148.44	108.48	142.52	135.97	137.28
Mean chromatin length/chromosome		5.97	5.82	4.26	4.24	5.32	7.42	5.42	7.13	6.80	6.86

t = terminal; st = subterminal; m = median; sm = submedian.

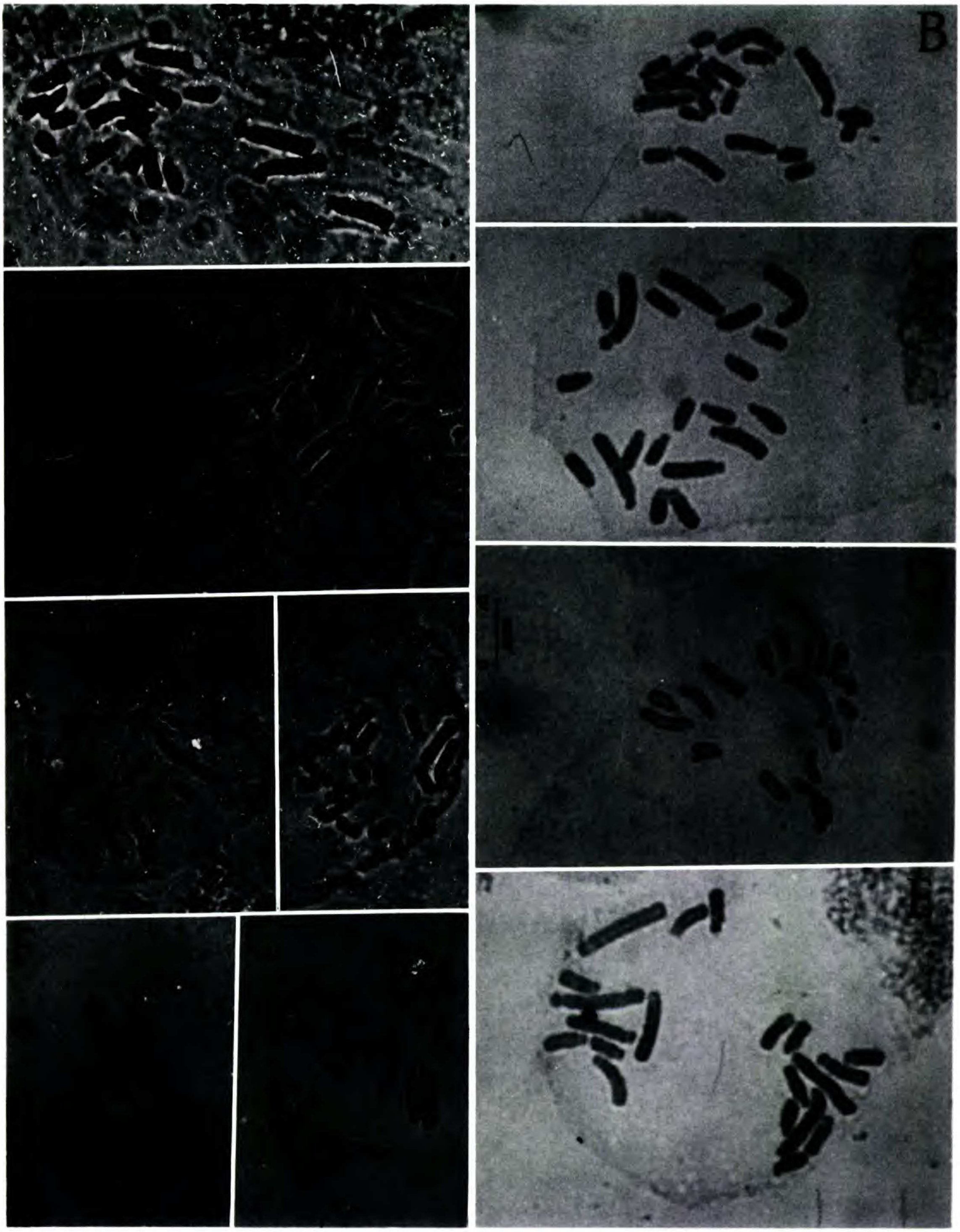

FIGURE 2. Somatic metaphase complements of the various groups. Bar represents 10 μm.

karyotypes (A_1–A_4) that fall into two sets. A_1 and A_2 are similar, forming one set; A_3 and A_4 are similar, forming the other set (Fig. 3, Table 1). Type A_1 represents plants of four contiguous sampling sites; A_2 represents plants of another two contiguous sampling sites about 400 km to the east of A_1. A_3 and A_4 represent separately each of the only two individual plants collected

in one sampling site about 80 km to the east of A_1.

A_1 karyotype consists of chromosomes whose average lengths vary between 3.67 μm and 11.0 μm, with an average total chromatin length of 119.34 μm per complement. The complement consists of three long, six medium, and one short pairs of chromosomes, all with terminal and subterminal centromeres. Endomitosis frequently occurs in the root cells. Meiosis is normal, and 10 bivalents are regularly formed.

Even though each complement of A_2, A_3 and A_4 could be resolved into pairs of similar chromosomes, members of such pairs are by no means identical. None of the individuals of these three karyotypes has flowered since they were brought into cultivation; hence these hypothetical pairings could not be verified in actual meiotic pairing.

A_2 consists of chromosomes whose average lengths range from 3.31 μm to 10.56 μm, with an average chromatin length of 116.36 μm per complement. The complement consists of six long, 10 medium, and four short chromosomes, all with subterminal–terminal centromeres. The longest two of the chromosomes have a centric region as wide as the short chromosome arm length.

A_3 chromosomes range in average length from 2.5 μm to 7.5 μm, with an average chromatin length of 85.26 μm per somatic complement. There are five long chromosomes in the complement, four of which resolve into two pairs while the fifth is associable with a shorter chromosome. This long chromosome has a conspicuous secondary constriction (arrowed in Fig. 2, A_3). The whole complement consists of five long, four medium, and 11 short chromosomes, all of which have their centromeres in the subterminal–terminal region, except the smallest two pairs which have submedian centromeres.

A_4 chromosomes vary in average length from 2.5 μm to 7.5 μm. They have an average chromatin length of 84.76 μm per somatic complement. The complement consists of four long, six medium, and 10 short chromosomes, all with subterminal–terminal centromeres except a short pair with median centromere.

Group B. This is represented by only one karyotype. Chromosomes vary in average length from 3.0 μm to 9.5 μm, with an average chromatin length of 106.36 μm per somatic complement. The complement consists of three long, three medium, and four short pairs. All the chromosomes have subterminal–terminal centromeres. The longest pair has a wide centric region. Meiosis is normal except for the infrequent early separation of members of a small pair.

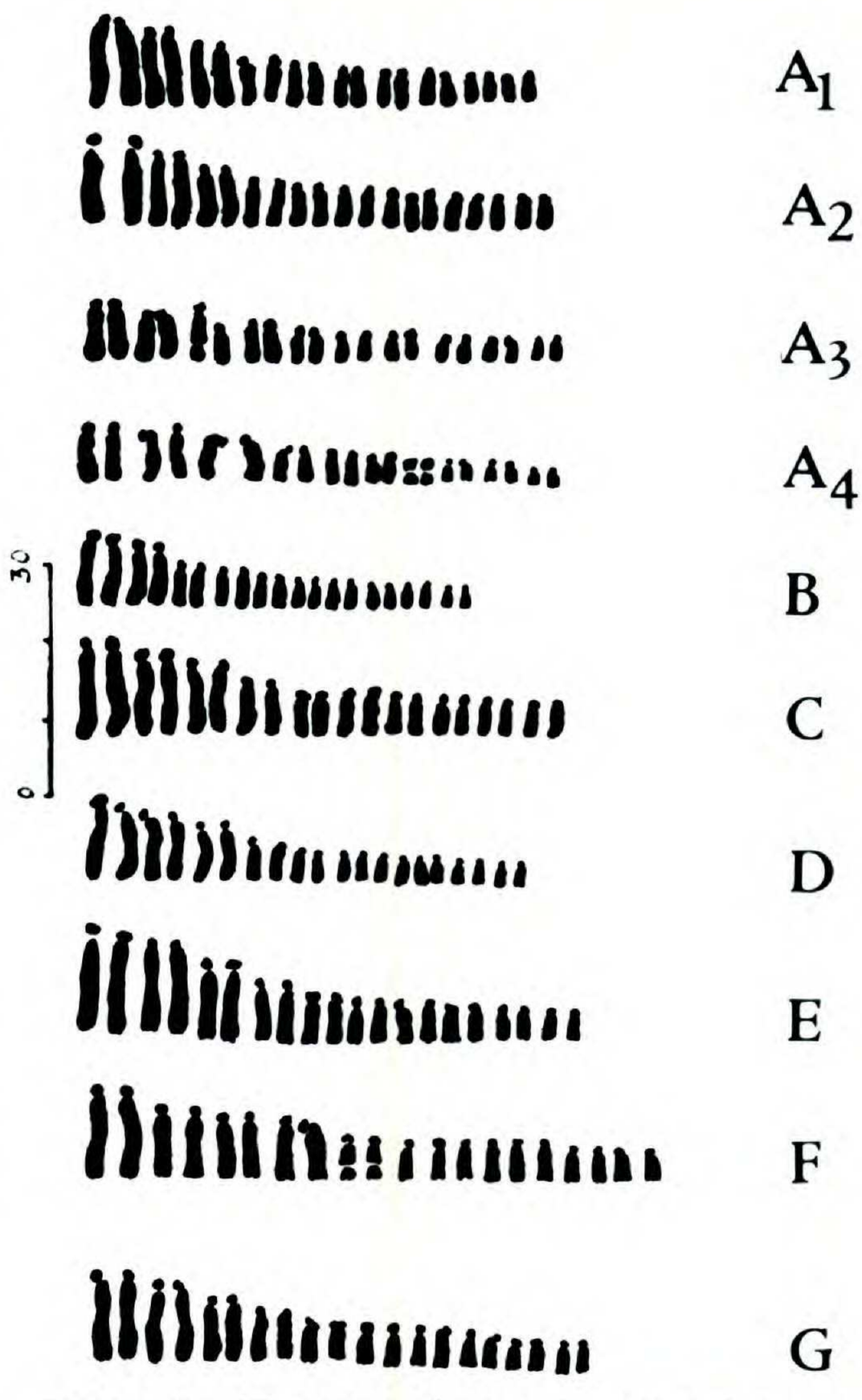

FIGURE 3. Idiograms of the various karyotypes represented by the groups. Horizontal bar represents 30 μm.

Group C. Chromosome length varies from an average of 4.88 μm to 13.1 μm, with an average chromatin length of 148.44 μm per somatic complement. There are five long and five medium pairs of chromosomes in the complement. All the chromosomes have subterminal–terminal centromeres. The longest pair has a centric region as wide as its short arm. Multivalents are frequently formed during meiosis.

Group D. Populations of this group are sympatric with those of Group B. The karyotype consists of chromosomes with subterminal–terminal centromeres. The average chromosome length varies from 3.5 μm to 9.82 μm, with an average chromatin length of 108.48 μm per somatic complement. There are three long, three medium, and four short pairs of chromosomes. The two

longest pairs have a wide centric region. Meiosis is regular.

Group E. The karyotype of this group consists of chromosomes with subterminal–terminal centromeres. Chromosomes vary in length from 4.25 μm to 13.5 μm and have an average chromatin length of 142.52 μm per somatic complement. There are four long and six medium pairs of chromosomes. The first and third long pairs have a wide centric region. Meiosis is regular.

Group F. The karyotype consists of chromosomes that vary in length from 4.1 μm to 12.05 μm, with an average chromatin length of 135.97 μm per somatic complement. The complement is made up of four long and six medium pairs, all with subterminal–terminal centromeres. The fifth pair has a secondary constriction on the long arm. Meiosis is regular.

Group G. The karyotype consists of chromosomes whose average lengths vary between 4.14 μm and 12.33 μm, with an average chromatin length of 137.28 μm per somatic complement. The complement consists of four long and six medium pairs, all with subterminal–terminal centromeres. Meiosis is regular.

DISCUSSION

The similarity in the morphology of the karyotypes is obvious. The differences in the karyomorphology of the different populations seem minute, but they are basic and do underlie the differences in the external morphology of each population. Even under cultivation for several years, these morphological differences are still retained. It is, however, evident that changes have occurred (or are occurring) in this taxon that may be correlated with the morphological differentiation of the populations. The recognition of different karyotypes that correspond to different morphological forms is noteworthy; the presence of more than one karyotype in an otherwise morphologically uniform unit raises interesting questions about evolutionary phenomena. This morphologically uniform unit, Group A, is interesting. Karyomorphological segmentation is not accompanied by external morphological differentiation, but it is partly correlated by habitat preferences; the A_2, A_3 and A_4 complements clearly demonstrate lack of homology in the morphology of the chromosomes. There is therefore enough evidence to suspect that these three complements represent natural hybrid swarms that introgressively identify with A_1, which is likely to be one of the putative parents. The different karyotypes are correlated with differences in external morphology and ecological preferences. Those forms that inhabit different ecological niches may have differentiated in response to differences in ecological demands, while those which inhabit same or similar ecologic niches must be fundamentally different genetically in order to retain their individual identities morphologically. In both cases, karyotype differentiation seems to have resulted in reproductive isolation by which the different forms are maintained in nature.

The differences in the amount of chromatin material may have resulted from (or led to) karyotype differentiation. Difference in the chromatin material is correlated with both morphological differentiation and ecological preferences (note A_3 and A_4, B and D are in the same niches; A_1 and A_2, and E, F, and G are in similar niches; and C and E are in different niches).

The morphological variability of this species has long been recognized, but no prior attempts have been made to distinguish the different forms beyond the arbitrary categorization of "large" and "dwarf." This is probably due to the fact that no field collection ever contains both floral and vegetative features together, as well as to the wide east–west distribution of the species. The aggressive exploitation of different niches by different biotypes has resulted in the differentiation of the morphological forms within the species' broad areas of distribution. The isolation of specific biotypes, forming small clusters of individuals, in such ecological niches probably led to the accumulation of favored genes and/or modest chromosomal changes and eventually to the specific karyotypes that are associated with specific morphological forms as well as with specific ecologic niches (Wright, 1940; Bush et al., 1977; Bengtsson, 1980). There is no doubt that morphological differentiation in these populations is more obvious than differentiation in chromosome morphology, suggesting that chromosome repatterning may have been mild and might have involved only small segments in gene/gene block rearrangement. Hence this species seems to comprise a stable polymorphism in which the different forms have attained reproductive isolation and genetic stability, and hence each form has retained its morphological identity. This case is therefore different from that of *Agrostis tenuis*

(Bradshaw, 1959) or *Elymus rechingeri* (Heneen & Runemark, 1962) but seems to be similar to that of the diploid neospecies of *Clarkia* (Lewis, 1973).

Flower formation and fruit development are a common feature with most of the morphological forms, especially in nature. Preliminary studies of meiotic behavior have shown regularity of pollen formation in most of them under cultivation. However, this apparent sexual reproduction is coupled with vigorous vegetative propagation by axillary bulb formation in varying degrees in all forms. It is therefore necessary to have a closer look at the reproductive biology of the entire species in order to ascertain the extent of actual sexual reproduction and the mechanism of pollination in each form. Hybridization experiments between the different forms, which will hopefully shed light on their genetic divergence, are in progress. Thus far, preliminary results of such experiments show successful artificial crossing between only two forms, A_1 and E.

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